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Acquired Hypoprothrombinemia: Effects of Danazol® Treatment

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The lupus anticoagulant may be accompanied by an acquired factor II deficiency and bleeding. We report on a patient with a lupus anticoagulant and factor II (FII) deficiency responsive to Danazol®. Acquired hypoprothrombinemia (FII) with the lupus anticoagulant (LA) may be accompanied by a hemorrhagic diathesis. A 64-year-old male with discoid lupus erythematosis bled after an intestinal polypectomy. His FII level was 18%, and his FII antigen level was 20%. Danazol® (D) (600 mg per day) administration was associated with a rise in FII activity and antigen to 50% within 10 days. The patient underwent abdominal surgery. We studied the effect(s) of D on the FII level and on other coagulation factors in this patient. The patient's plasma FII antigen had a single precipitin arc compared to the two peaks of normal plasma on counterimmunoelectrophoresis with Ca^{++}. The samples pre- and during D therapy had the same positively charged arc as normal samples, although they were quantitatively different. Neuraminidase treatment demonstrated a decrease in the positively charged migration of normal and the patient's FII antigen. Affinity chromatography of normal and patient plasma on a Sepharose protein A column revealed FII antigen present in the patient's bound fraction. The relative percentages of bound FII before and during D treatment were similar. During D therapy, levels of FIX and X rose 50–100%, and protein C rose 20–25%, while free protein S did not change. D is an effective therapy for acquired FII deficiency associated with LA. D does not affect the binding of Ig to FII, but D raises FII levels by increasing synthesis of the FII protein.

Key words: hypoprothrombinemia, lupus anticoagulant, discoid lupus erythematosus, Danazol®, factor II, factor IX, factor X, protein C, protein S

INTRODUCTION

The lupus anticoagulant (LA) is detected in approximately 10–15% of patients with systemic lupus erythematosus (SLE) [1]. LA is directed against phospholipids and is identified by a prolonged activated partial thromboplastin time or other coagulation tests that require phospholipids for clot formation. Patients with the lupus anticoagulant who have a prolonged prothrombin time (PT) usually have a deficiency of prothrombin (factor II) antigen [1,2], with the level of antigen reduced to the same extent as the activity. The plasma of patients with factor II deficiency associated with the lupus anticoagulant do not contain an anticoagulant capable of neutralizing prothrombin in vitro [3]. The proposed mechanism of the decrease in prothrombin antigen and activity is the rapid clearance of complexes of prothrombin with an antiprothrombin antibody that does not neutralize the in vitro activity of prothrombin [3–5]. These antibodies only react with epitopes on the carboxy-terminal portion of the factor II molecule [4]. Most patients with LA do not have abnormal bleeding; however, those patients with LA who do bleed often have a decreased factor II, thrombocytopenia, or other hemostatic defect(s). Adrenal corticosteroid therapy is usually effective in correcting acquired factor II deficiency [5–7].

CASE REPORT

We report on a 64-year-old male with discoid erythematosus, LA, and factor II deficiency, who experienced

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TABLE I. Initial Laboratory Data of Patient*

	Normal	Patient (3/18/81)
Prothrombin time (sec)	<13.2	16.5
Partial thromboplastin time (sec)	<38.6	43.0
Thrombin time (sec)	<35.0	29.5
Fibrinogen (mg/dl)	186–354	425.0
Platelet count (10^3/ul)	181–386	165.0
Factor II activity (%)	52–141	22%
Factor II antigen (%)	54–161	18%
Factor V (%)	47–154	48%
Factor VIII (%)	41–152	63%
Factor VII (%)	48–144	52%
Factor IX (%)	51–153	70%

*Note that the patient's coagulation factor values are in the lower range of normal; however, when factor II activity started to rise, some of the other coagulation factors also rose to normal or elevated levels.

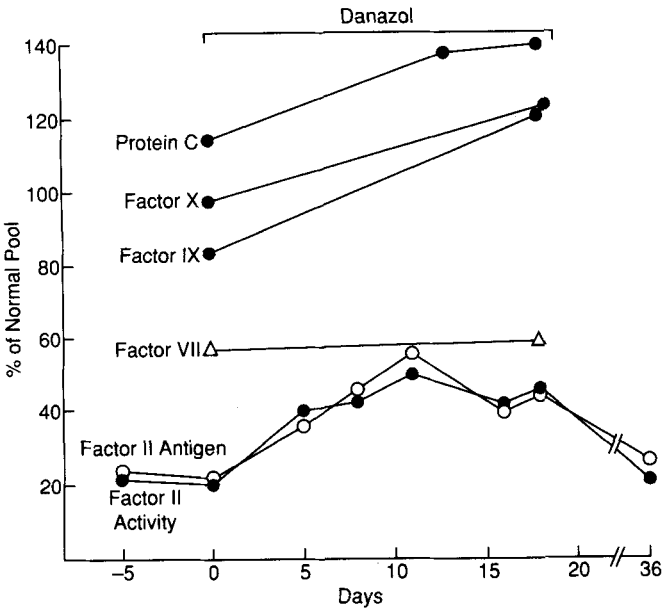

Fig. 1. Graph shows rise in protein C, factor X, factor IX, and factor II antigen and activity over days, with the patient receiving 600 mg of Danazol® per day.

postsurgical bleeding. His factor II level did not respond to corticosteroid therapy. In this study we investigated this patient's factor II deficiency and the beneficial effects of Danazol® therapy on his factor II deficiency. The patient had skin rashes for 22 years which were aggravated by sunlight. He has also experienced arthralgias in his hands and feet. In 1967, he was diagnosed as having discoid lupus erythematosus. In the early 1970's, he noticed that when he used aspirin, he had frequent epistaxis. He stopped the use of all medications. In late 1980, he experienced episodes of rectal bleeding. In 1981, he had two benign colonic polyps removed by colonoscopy. Twenty-four hr later he required hospitalization for gastrointestinal hemorrhage. He was treated with plasma infusions and intravenous Pitocin®. Because of his abnormal coagulation studies, he was referred to the NIH. When seen, the patient was asymptomatic; the initial laboratory data are presented in Table I. Even though the patient's PT was shortened with Danazol®, it never reached a normal level. We believe that the prothrombin time prolongation was due to the presence of the lupus inhibitor. The dilute tissue thromboplastin inhibition test has been positive in this patient from 1981 to present (1995).

In 1982 and 1988, he required surgery for a large inguinal hernia. In 1982 he received Prednisone, 30 mg per day, for 2 weeks without any effect on his factor II level. Because of his failure to respond to Prednisone, in 1982 and 1988, he received Danazol®, 200 mg three times a day.

Danazol®-induced changes in his coagulation tests (from 1981, during a trial of Danazol® therapy) are depicted in Figure 1 [10]. Other pertinent laboratory data from 1987–1988 include a biologic false-positive serological test for syphilis, normal C_3 and C_4 levels, negative anti-DNA titer, ANA titer 1:160, diffuse and speckled pattern, and high-titer IgG and IgM anti-cardiolipin antibodies.

MATERIALS AND METHODS

All blood for coagulation studies was collected in polypropylene tubes containing sodium citrate (final concentration 10.9 mM) by a two-syringe technique. Plasma was separated and used immediately for assays, or kept frozen at −70°C and assayed at a later date.

Factor II activity was measured using Taipan snake venom [13]. Factor II antigen was measured by the electroimmunoassay of Laurell [12]. A monospecific rabbit anti-human prothrombin antibody was employed for the Laurell and the CIE. Counterimmunoelectrophoresis (CIE) of plasma prothrombin was performed as previously described with Ca lactate or with EDTA [2,8].

Clostridium perfringens neuraminidase was used to treat plasma samples prior to CIE. The neuraminidase, 1.52 U/ml (Sigma Chemical Co., St Louis, MO), was incubated with plasma samples at 37°C for 4 hr prior to electrophoresis.

Purification of patient and normal IgG was performed on Sepharose protein A columns (Pierce Chemicals, Rockford, IL). The prepared columns were equilibrated with Pierce® ImmunoPure IgG binding buffer. Two ml of citrated plasma were mixed with the binding buffer, centrifuged, and then run on the column according to the manufacturer.

The IgG was eluted with the Pierce® ImmunoPure IgG elution buffer. An aliquot of the bound and unbound plasma fractions were tested for factor II activity and antigen content in the Laurell assay with a monospecific rabbit anti-prothrombin antibody (see above).

The protein C and protein S levels were determined at the University of Oklahoma. Protein C was determined by

an immunological method. The normal range is 65–150%. Protein S was determined by radioimmunoassay, and it measured both total and free protein S. The normal range for total proteins was 18.9 ± 6.0 μg/ml, and for free proteins 5.9 ± 2.4 μg/ml.

RESULTS

On two different occasions (1982 and 1988), the patient received 600 mg of Danazol® per day. On each occasion, his factor II activity rose to a peak value of 45–53%, with a concomitant rise in his factor II antigen. Figure 1 shows the coagulation response to Danazol® therapy in this patient. Factor II activity and antigen rose within 5 days after initiation of Danazol® therapy and reached a plateau at day 8. Moderate increases in factors IX, X, and protein C were also observed, while factor VII and free protein S levels (not shown) did not change.

While receiving Danazol®, the patient underwent two separate abdominal surgical procedures without abnormal bleeding. The only observed toxicity was on one occasion when the patient had a mild nonsymptomatic elevation of his hepatic enymes. This occurred at a time that would have been consistent with non-A, non-B hepatitis associated with his prior plasma transfusions.

Examination of the patient's plasma factor II revealed significant differences compared to normal factor II antigen. The CIE of normal factor II (Fig. 2) shows two peaks. The first and slowest-migrating peak is the functional peak which binds to calcium; the second peak does not bind to calcium. The patient's prothrombin with and without Danazol® bound to calcium, but not was well as the normal first peak. This may be due to the presence of attached antibody.

Figure 4 shows the factor II arcs of the patient. Normal plasma arcs remained the same as in Figure 2, except that they were shifted cathodally.

Neuraminidase removes the terminal sialic acid from the carbohydrate side chain of the molecules, thereby altering the charge and causing the shift in electrophorectic mobility. Normal and patient plasma reacted the same, suggesting that sialic acid is not responsible for the abnormality in the patient arc.

Figure 3 shows the CIE arcs without Ca^{++} in the buffer, but with the presence of EDTA. The normal prothrombin has one peak. The patient had one peak before Danazol® therapy that is shifted toward the anode, possibly due to the immunoglobulin bound to the prothrombin. CIE migration is determined by size and charge. The patient's prothrombin, while receiving Danazol®, does not have increased immunoglobulin levels and returns to a normal pattern.

Both patient and normal plasma were passed over protein A-Sepharose columns. Equivalent amounts of normal and patient Ig bound to the columns, as judged by the

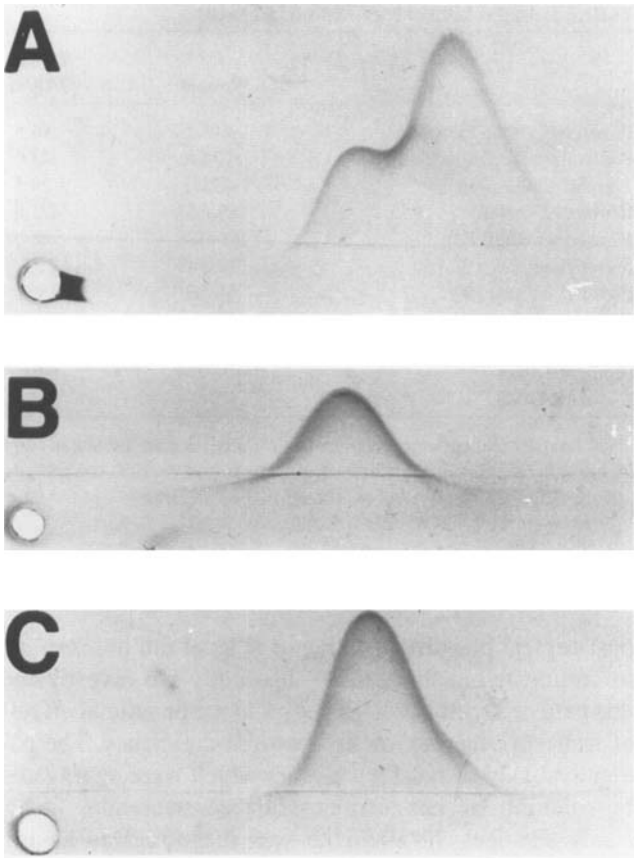

Fig. 2. CIE with 2 mM Ca^{++} A: Normal plasma. B: Patient plasma before Danazol®. C: Patient plasma during Danazol®. Anti-factor II antibody was used.

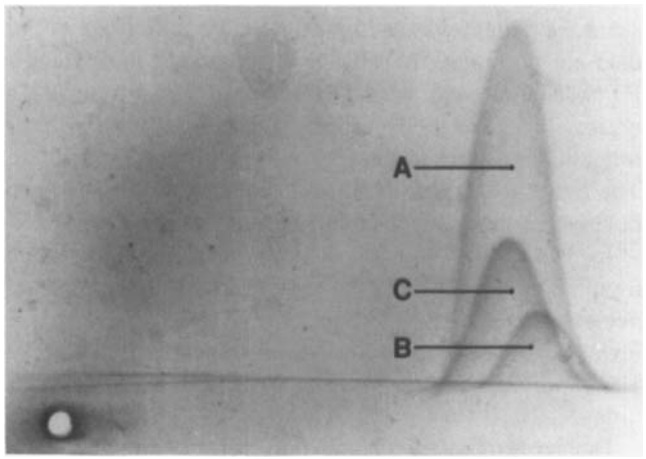

Fig. 3. CIE. A: Normal plasma. B: Patient plasma. C: Patient plasma during Danasol®. EDTA is incorporated into the gel. Patient plasmas are shifted anodally compared to normal plasma. Plasma taken while the patient was on Danazol® appears to be shifting back toward normal peak.

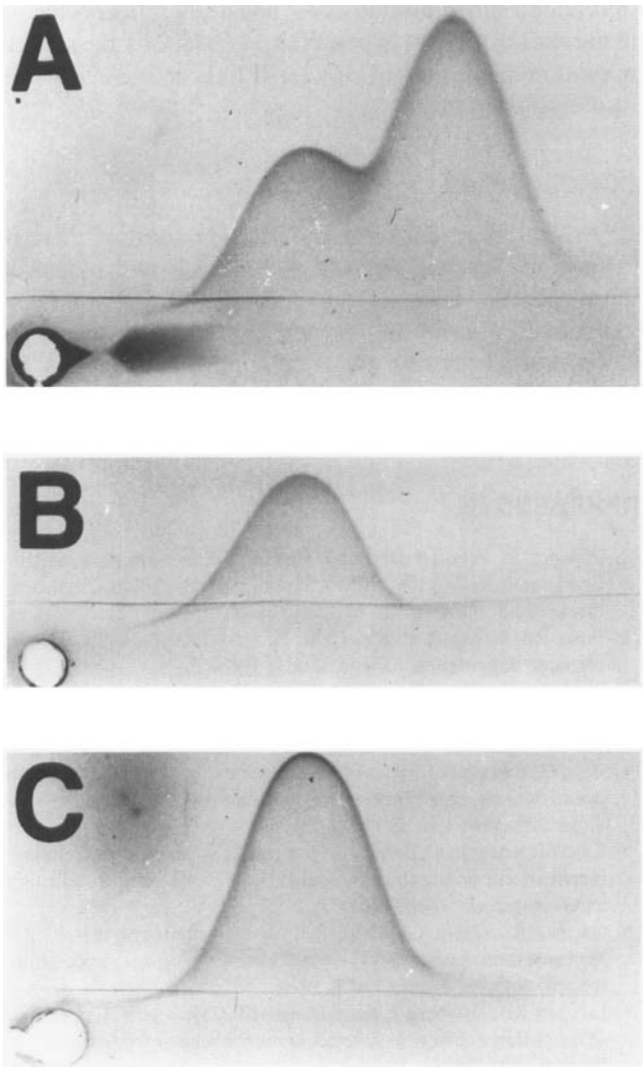

Fig. 4. CIE with 2 mM Ca^{++}. A: Normal plasma. B: Patient plasma before Danazol®. C: Patient plasma during Danazol®. Each plasma was treated with neuraminidase (1.52 U/ml) at 37°C for 4 h. Peaks were similar to peaks in Figure 2, except that they were all shifted cathodally the same amount.

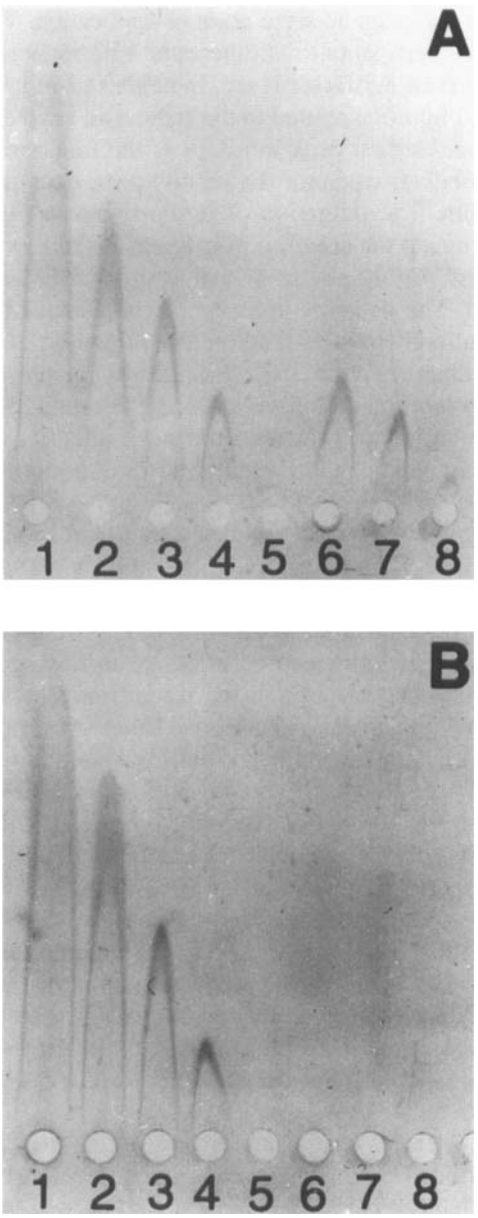

Fig. 5. Laurells were made with antibodies to prothrombin. Samples were from the protein A-sepharose columns. The protein A column was used to pull out antibodies plus anything bound to them. (A) Patient plasma showed factor II bound to normal IgG; (B) normal plasma showed no factor II bound to normal IgG. The first four lanes are controls for prothrombin.

absorbance at 280 nm. When the bound material was eluted and identical column fractions from normal and patient plasma were analyzed for factor II antigen by Laurell assay (Fig. 5), normal immunogloblin had no prothrombin attached, whereas the patient's immunoglobulin had prothrombin attached to it. This would indicate that some of the patient's antibody was to prothrombin instead of antiphospholipid.

DISCUSSION

This patient had discoid lupus erythematosus, LA, and acquired hypoprothrombinemia. His factor II antigen level was decreased to the same level as his factor II activity. Neither his plasma nor his purified IgG inhibited his factor II activity. In these ways the patient was similar to other individuals previously reported with this syndrome; however, the appearance of his factor II arc in counterimmunoelectrophoresis was quite different from the descriptions, in previous publications [2,8]. Particularly in terms of Ca^{++}, our patient's factor II arc corresponded only to the most cathodal portion of the normal arc, and it lacked the previously described slow-migrating

shoulder and peak near the point of application. We have seen one other patient with the same electrophoretic abnormalities of her factor II arc. In neither patient was the aberrant migration related to the sialic acid content of the factor II. The first peak in a CIE is the functional peak which binds to calcium; the second peak does not bind to calcium. The migration of prothrombin in Figures 2 and 4 is due to the presence of calcium. Asialo prothrombin plus CA^{++} is also responsible for the migration in Figure 4. The patient's prothrombin is aberrant because of the antibody to prothrombin. The migration of patient prothrombin in Figure 3 is influenced by the presence of the antiprothrombin antibody. During Danazol® therapy, more prothrombin is released and less antibody is bound. Therefore, prothrombin returns toward a normal migration.

The patient's factor II level did not respond to a 1-week trial of corticosteroids. Because the patient was going to require elective surgery, we examined other methods for raising his factor II level.

Danazol® is known to be effective in raising the CI esterase inhibitor level and the α1-anti-trypsin level in hereditary angioedema. Laurell and Rannevik [9] showed that in women receiving Danazol® for endometriosis, the factor II antigen level increased 50% within 3 weeks to 97% of normal or 147%. Our patient received Danazol® on three occasions, and on each, his factor II activity and antigen increased to a level of 60%. While the patient was taking Danazol®, the factor II antigen still showed only a cathodal peak. During this time, his prothrombin time and activated partial thrmoboplastin time were shortened 2.5 and 8.0 sec, respectively, yet the ratio of the patient plasma to control plasma in the dilute tissue thromboplastin inhibition test did not significantly change (ratio, 2–2.5).

Corticosteroids have been effective in the treatment of the acquired factor II deficiency associated with LA [5–7]. The mechanism is believed to be due to decreased clearance of the factor II complex in the early phase of treatment [4,5], and possibly to decreasing antibody synthesis later in the course of treatment. Although we do not have any kinetic data, our results indicate that Danazol® raises the level of factor II by increasing its synthesis, and there is no significant effect on antibody synthesis, as judged by the constant level of LA measured in the dilute tissue thromboplastin inhibition assay, and no significant change in the antibody factor II complex, as judged by the similar migration of the patient's factor II CIE before and while receiving Danazol®.

CONCLUSIONS

Danazol® appears to be an effective form of therapy to increase factor II in patients with LA and hypoprothrombinemia. Danazol® may also be useful for those individuals who do not respond to corticosteroids, or in whom corticosteroids are counterindicated. Factors IX and X and protein C also rose while the patient was treated with Danazol®.

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